COMPARATIVE STIMULATION OF PARTHENOCARPY IN THE TOMATO BY VARIOUS INDOLE COMPOUNDS[1]

H. M. SELL, S. H. WITTWER, T. L. REBSTOCK AND C. T. REDEMANN

DEPARTMENTS OF AGRICULTURAL CHEMISTRY AND HORTICULTURE,
MICHIGAN STATE COLLEGE, EAST LANSING, MICHIGAN

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The stimulatory effect of indole compounds on fruit-setting of several horticultural crops (**7, 8, 18**) was soon established following the identification of indole-3-acetic acid as a plant growth hormone (**12**). Strangely enough, however, for the stimulation of parthenocarpy many related compounds, some analogs of indole-3-acetic acid, were found more effective (**7, 8, 11, 23, 25, 27, 32**). Meanwhile, extracts of corn kernels in the milk stage were described as having a fruit-setting potency much greater than could be accredited to indole-3-acetic acid alone (**9, 26**). Recently the fruit-setting factor derived from the ethanol extracts of immature corn kernels was characterized by REDEMANN *et al.* (**16**) as the ethyl ester of indole-3-acetic acid and was found to induce parthenocarpic fruit development in the tomato when applied in dilutions of 1 part in 10,000. Thus, the fruit-setting activity was multiplied almost 100-fold compared with the free acid or the salts of heteroauxin.

Recent studies concerned with the relation of chemical structure to activity, the relative instability of indole-3-acetic acid, the enhancement of activity by the addition of chlorine to the ring structure of certain growth substances, the role of growth substances in *in vitro* culture of tomato fruits (**14**) and finally, the scant attention directed toward halogenated or otherwise substituted indole compounds in biological activity testing suggested a more detailed evaluation of this chemical group of compounds with respect to the comparative induction of parthenocarpic fruit development in the tomato.

Methods

A known quantity of the compound was dissolved in anhydrous, peroxide-free ether and diluted to the desired concentration. An aliquot of this solution containing the required amount of the compound was added to a known quantity of anhydrous lanolin and stirred until solution was complete. The ether was removed by immersing the containers of the solution in a hot water bath. For higher dilutions the same procedure was followed with smaller aliquots.

Estimation of the fruit-setting activity of the various indole compounds consisted of applying 1, 0.1, 0.01, and 0.001% lanolin solutions of each chemical to the ovary of the tomato flower from which the stamens had been

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removed. From 15 to 20 milligrams of lanolin containing the desired chemical were applied to each ovary. About 12 blossoms from the first flower clusters of three to four tomato plants (variety, Michigan State Forcing) of comparable physiological and nutritional status were employed for each dilution. The dilutions which caused definite fruit development of the emasculated blossoms were determined. The relative effectiveness of a particular substance at the various concentrations is indicated (table I) by the number of plus signs allotted. Three plus signs signify high activity with 100% set and fruit development equivalent or superior to natural pollination, two signs moderate activity with about 50% fruit set but with fruit size somewhat less than from pollination, and one plus sign slight but definite growth apparent in 10 to 25% of the ovaries treated.

The following compounds were obtained commercially: Indole-3-acetic acid, β(indole-3)-*n*-proprionic acid, γ(indole-3)-*n*-butyric acid, indole, isatin, L-tryptophan, and tryptamine from the Eastman Kodak Company, 3-methyl indole from the Matheson Company, and 2,3-diphenylindole from the B. F. Goodrich Chemical Company. The 4-chloroindole-3-acetic, 5-chloroindole-3-acetic, 6-chloroindole-3-acetic and 7-chloroindole-3-acetic acids were supplied by Dr. Sidney W. Fox of Iowa State College at Ames, Iowa. The 1-benzoylindole-3-acetic and 2-phenylindole-3-acetic acids were supplied by Mr. W. F. Houff of Michigan State College. The following compounds were prepared according to procedures given in the literature: methylindole-3-acetate, ethylindole-3-acetate, *n*-propylindole-3-acetate, *n*-butylindole-3-indoleacetate, *n*-amylindole-3-acetate (**17**), ethyl-5-chloroindole-3-acetate, 5,7-dichloroindole-3-acetic acid (**6**), 4,7-dichloro-2-methylindole-3-acetic acid (**10**), indole-3-pyruvic acid (**3**), indole-3-ethanol (**20**), 2-methylindole-3-acetic acid (**21**), 2-methylindole (**5**), indole-3-aldehyde (**19**), oxindole (**22**), and indole-3-carboxylic acid (**28**).

Results

The comparative effectiveness of various indole compounds at different concentrations to induce parthenocarpy in the tomato is given in table I. The methyl and ethyl esters of indole-3-acetic acid were approximately 100 times more effective than indole-3-acetic acid and were the only compounds tested having activity at a solution concentration of 0.001% in lanolin. The activity of the esters decreased as the size of the alkyl radical was increased with the exception of the methyl and ethyl esters which had identical activity. The introduction of chlorine on the benzene ring of indole-3-acetic acid in either the 4, 5, 6, or 7 position enhanced the activity 10 times above that of the free acid. However, the chloroindole-3-acetic acids were decidedly effective only at dilutions of 0.01%, and thus, were only one tenth as effective as ethylindole-3-acetate. Esterification of the chloroindole-3-acetic acids did not increase the activity to induce parthenocarpy as it did in the case of indole-3-acetic acid. The position of the chlorine in the benzene ring had no effect as far as fruit-setting effectiveness was concerned.

However, the addition of two chlorine atoms on the ring (5,7-dichloroindole-3-acetic acid) slightly reduced rather than enhanced the activity. Some effects on the fruit-setting response obtained by lengthening the alkyl radical of the indole acids has already been reported (**7, 8, 11, 23**).

Indole-3-pyruvic acid, indole-3-ethanol, and indole-3-nitrile induced fruit-setting only at 1% solutions. The introduction of a benzoyl radical

TABLE I

RELATIVE EFFECTIVENESS OF INDOLE COMPOUNDS IN LANOLIN ON THE STIMULATION OF PARTHENOCARPY IN THE TOMATO.

Compounds	Concentration of solutions			
	1.0%	0.1%	0.01%	0.001%
Indole-3-acetic acid	+++*	+++	+	Inactive
1-Benzoylindole-3-acetic acid	+++	Inactive	Inactive	Inactive
2-Methylindole-3-acetic acid	+++	Inactive	Inactive	Inactive
2-Phenylindole-3-acetic acid	+	Inactive	Inactive	Inactive
B-(Indole-3)-*n*-propionic acid	+++	Inactive	Inactive	Inactive
γ-(Indole-3)-*n*-butyric acid	+++	+++	++	Inactive
Indole-3-pyruvic acid	+++	Inactive	Inactive	Inactive
Methylindole-3-acetate	+++	+++	+++	++
Ethylindole-3-acetate	+++	+++	+++	++
n-Propylindole-3-acetate	+++	+++	+++	Inactive
n-Butylindole-3-acetate	+++	+++	++	Inactive
n-Amylindole-3-acetate	+++	+++	Inactive	Inactive
4-Chloroindole-3-acetic acid	+++	+++	+++	Inactive
5-Chloroindole-3-acetic acid	+++	+++	+++	Inactive
6-Chloroindole-3-acetic acid	+++	+++	+++	Inactive
7-Chloroindole-3-acetic acid	+++	+++	+++	Inactive
Ethyl-5-chloroindole-3-acetate	+++	+++	+++	Inactive
5,7-Dichloroindole-3-acetic acid	+++	+++	+	Inactive
4,7-Dichloro-2-methylindole-3-acetic acid	Inactive	Inactive	Inactive	Inactive
Indole-3-ethanol	+++	Inactive	Inactive	Inactive
Indole-3-nitrile	+	Inactive	Inactive	Inactive
Isatin	Inactive	Inactive	Inactive	Inactive
L-Tryptophan	Inactive	Inactive	Inactive	Inactive
Indole-3-aldehyde	Inactive	Inactive	Inactive	Inactive
Oxindole	Inactive	Inactive	Inactive	Inactive
2,3-Diphenylindole	Inactive	Inactive	Inactive	Inactive
Tryptamine	Inactive	Inactive	Inactive	Inactive

*The number of plus signs at each concentration indicates the relative magnitude of activity of each substance.

in position 1 or a methyl group on position 2 of indole-3-acetic acid reduced the fruit-setting activity to approximately one twentieth of the free acid. With the substitution of a phenyl radical in the 2 position of the indole nucleus, the activity was less than that of the 2-methylindole-3-acetic acid. This indicates that the 2 position must be left unsubstituted to obtain maximum efficiency. The introduction of a chlorine atom in position 4 as in 4,7-dichloro-2-methylindole-3-acetic acid destroyed the activity completely. This demonstrates that additional substitution on the benzene ring of

2-methylindole-3-acetic and especially in position 4 (ortho) eliminated entirely the fruit-setting activity of the compound.

Indole, isatin, 2-methylindole, 3-methylindole, L-tryptophan, indole-3-aldehyde, oxindole, 2,3-diphenylindole, indole-3-carboxylic acid, and tryptamine were inactive.

Discussion

The indole compounds occupy a unique position in that the only naturally occurring growth substance, the existence and identity of which is beyond dispute, is indole-3-acetic acid (heteroauxin) or its ethyl ester (**16**). It may now be safely assumed that indole-3-acetic acid is common to all green plants. In view of this, it is a striking fact that halogenated and otherwise substituted indole compounds have received only cursory attention; yet, highly active substances might logically be found among them.

In the present studies at least 10 indole compounds, specifically esters and chlorinated derivatives of indole-3-acetic acid, were found more effective than indole-3-acetic acid itself for stimulating parthenocarpic fruit development in the tomato. That a number of derivatives of indole-3-acetic acid, particularly the esters, may be more active than the unsubstituted acid in some plant growth responses has already been reported (**1, 29, 30, 31**), conversely, considerable evidence has been accumulated by the *Avena* and other biological assays that all substitutions so far studied do not increase and may greatly reduce the activity of indole-3-acetic acid (**2, 4, 13, 15, 24**). The present work on the enhancement of tomato parthenocarpic induction by chlorine substitution and esterification of indole-3-acetic acid interestingly demonstrates a parallel with some of the phenoxy acids, and the need for thorough testing by a number of assay methods including intact plants such as the tomato before evaluating the relative activity of a plant growth-regulator. It is conceivable that derivatives of even greater activity from the standpoint of fruit set might exist among the indole compounds.

The ortho effect in plant growth-regulators as reported by HANSCH and MUIR (**10**) and criticized by ZIMMERMAN and HITCHCOCK (**33**) and THIMANN (**25**) was observed in the fruit-setting responses induced by variously substituted indole compounds. Substitutions of the methyl or phenyl radical in the 2 position greatly reduced the activity, and substituents in both ortho positions as in 4,7-dichloro-2-methylindole-3-acetic acid completely eliminated activity, although 4-chloroindole-3-acetic acid was found more active than the parent compound. All fruit-setting responses could not, however, be explained by the ortho effect theory an example being the weak activity obtained with 1-benzoylindole-3-acetic acid.

Summary

In the tomato parthenocarpic fruit test, 10 derivatives of indole-3-acetic acid were found to have greater fruit-setting activity than the parent compound. Esterification of the carboxyl group of indole-3-acetic acid with a

methyl or ethyl radical increased the activity 100-fold. The activity of the esters decreased as the size of the alkyl radical was increased. Mono-substitution of the hydrogen by chlorine in any of the positions in the benzene ring of indole-3-acetic acid increased the activity 10-fold, and resulted in indole compounds one tenth as effective as methyl or ethylindole-3-acetate. In contrast to indole-3-acetic acid, esterification of the chloroindole-3-acetic acids did not increase the fruit-setting activity. The 1-benzoylindole-3-acetic acid and 2-methylindole-3-acetic acid were only one tenth as effective as the parent compound. Substitution of a phenyl group for a methyl radical in the 2 position further decreased the activity, and substituents in both positions ortho to the alkyl side chain completely eliminated activity.

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